Edible phosphoprotein film

FIELD OF THE INVENTION

The present invention generally relates to films, and more particularly to edible films. More specifically, the present invention relates to edible film compositions comprising a phosphoprotein preparation, and to methods of making the same.

BACKGROUND OF THE INVENTION

Oral cleansing and breath freshening may be difficult or inconvenient at times, depending on the nature of the breath freshening desired and the situation in which the breath freshening must be carried out. Methods such as brushing, flossing and gargling using various devices and compositions are common oral care practices that are suitable for carrying out in the privacy of one's home.

However, such devices and compositions are less convenient to use away from the home where bathroom facilities may be unavailable or unsanitary. Less obtrusive oral care products have been developed for use in these situations, such as breath-freshening chewing gums, lozenges, mouth sprays, and edible films.

A variety of edible films are known in the art.

For example, US patent 4,562,020 describes a self supporting glucan film prepared from an aqueous solution comprising pullulan or elsinan. These films are said to be useable as an edible, water-soluble packaging material.

US patent 4,623,394 describes edible films comprising pullulan and heteromannan, which are said to exhibit controlled disintegrability under hydrous conditions.

US patent 4,851,394 discloses an edible film based on the reaction product of a polyhydric alcohol and glucomannan and optionally other natural polysaccharides. These films are said to have desirable properties such as water resistance, heat resistance and strength.

European patent publication 1,008,343 A1 discloses a film preparation for oral drug delivery and said to have rapid solubility, comprising a drug, an edible polymer and a saccharide.

US patent application publication No. 2002/0150544 A1 discloses an edible film containing therapeutic agents and/or breath freshening agents for use in the oral cavity, comprising a mixture of a water-soluble polymer, a polyalcohol, a cosmetically and/or pharmaceutically active agent such as a breath freshener, and a flavor.

US patent application publication No. 2003/008008 A1 discloses edible films having an antimicrobial effect, comprising a water-soluble polymer such as pullulan, and an essential oil selected from thymol, methyl salicylate, eucalyptol and menthol.

US patent application publication No. 2003/0035841 A1 describes an edible film for oral mucoadhesion, comprising at least three film-forming agents, namely a maltodextrin, a hydrocolloid and a filler, but not including pullulan.

US patent application publication No. 2002/131990 A1 also discloses pullulan-free edible films, comprising a film forming agent (such as carageenan), a bulk filler agent and a plasticizing agent.

However, despite the existence of rapidly soluble edible films in the prior art, there is still room for improvement in such films. For example, the available pullulan-based films have such high solubility that on contact with the consumer's mouth they may impart an undesirable "grabbing" sensation as they dissolve. Accordingly, the mouth feel on dissolution of such films may not be as clean as is ideal.

It is an object of the present invention to provide an edible film which goes at least some way towards overcoming the above disadvantage, or at least to provide the public with an a useful choice.

SUMMARY OF THE INVENTION

Accordingly, in a first aspect the present invention provides a film comprising:

(a) a phosphoprotein preparation, wherein the phosphoprotein preparation has been obtained by partially cross linking a partial hydrolysate of casein or a caseinate,

(b) a source of one or more physiologically acceptable cations, and

(c) a plasticizer.

In preferred embodiments, the film is edible.

In preferred embodiments, the film is substantially soluble.

In preferred embodiments, the cations are divalent.

In preferred embodiments, the source of the cations comprises a substantially water-insoluble salt of the cations.

In preferred embodiments, the cations comprise calcium ions.

In particularly preferred embodiments, the source of calcium ions comprises calcium phosphate.

In certain preferred embodiments, some or all of the calcium phosphate is in the form of calcium hydroxyapatite.

In particularly preferred embodiments, the source of calcium comprises natural milk calcium phosphate, such as that available under the trade name ALAMIN®.

In preferred embodiments the plasticizer comprises a polyhydric alcohol, preferably glycerol.

In preferred embodiments, the partial hydrolysate of casein or a caseinate has been obtained by enzymatic hydrolysis of acid casein, rennet casein or a caseinate.

In preferred embodiments, the enzyme is trypsin, and the partial hydrolysis has been carried out at a pH of from about 7 to about 8.

In preferred embodiments, the partial cross linking has been carried out enzymatically, using transglutaminase, preferably at a pH of from about 7 to about 8.

In preferred embodiments, the edible film further comprises one or more emulsifiers. In one embodiment, the emulsifier comprises a citric acid ester of mono- and diglycerides, such as that commercially available under the trade name Lamegin 609ZE.

In further embodiments, the edible film may further include one or more additional agents such as flavoring or breath freshening agents, sweeteners, coloring agents, pH control agents and stabilizers.

In certain embodiments, the edible film may further include one or more active agents selected from the group consisting of an oral care agent, a pharmaceutical or veterinary agent, a nutraceutical agent, a salivary stimulant agent, a vitamin, a mineral and combinations thereof.

In a further aspect, the present invention provides a process of producing an edible film, the process comprising the steps of:

(a) processing a mixture comprising (a) a phosphoprotein preparation, wherein the phosphoprotein preparation has been obtained by partially cross linking a partial hydrolysate of casein or a caseinate, (b) a source of one or more physiologically acceptable cations, and (c) a plasticizer, to form a film; and

(b) optionally, drying the film to reduce the moisture content to a desired level.

In preferred embodiments, the mixture includes water.

In certain preferred embodiments, the processing includes the steps of applying the mixture to a surface followed by drying to form the film.

In other preferred embodiments, the processing comprises extruding the mixture to form a film.

In still a further aspect, the present invention provides a mixture capable of being processed to produce an edible film, the mixture comprising (a) a phosphoprotein preparation, wherein the phosphoprotein preparation has been obtained by partially cross linking a partial hydrolysate of

casein or a caseinate, (b) a source of one or more physiologically acceptable cations, and (c) a plasticizer.

Although the invention is broadly as defined above, it is not limited thereto and also includes embodiments of which the following detailed description provides examples.

DESCRIPTION OF THE INVENTION

The applicants have surprisingly found that thin, edible films, having desirable organoleptic and physical properties, and which dissolve rapidly in the mouth, can be prepared from certain phosphoproteins when combined with a suitable cation. The present invention therefore provides novel films, preferably edible films, and methods of making and using them.

The films of the present invention have a number of applications. For example, they may be used to deliver breath fresheners or other oral care agents. Alternatively or additionally, they may act as carriers for pharmaceutical or veterinary agents, nutraceutical agents, salivary stimulant agents, vitamins, minerals, or combinations thereof. In other embodiments, the edible films of the present invention may be used as a readily soluble and/or edible coating for foods. In still further embodiments, the films may be used as a vehicle to apply active agents topically to the skin.

The films of the present invention, although still dissolving rapidly in the mouth, at least in certain preferred embodiments, have a lower solubility than certain pullulan-based films that are currently available. This in turn means that at least in certain preferred embodiments, the edible films of the present invention have the advantage of "grabbing" less in the mouth and providing a relatively clean mouth feel on dissolution.

Accordingly, the films of the present invention are preferably substantially soluble. As used herein, the term "substantially soluble" means that the film as a whole disintegrates and dissolves readily in the mouth of a consumer. However, it will be understood that some components of the film, in particular salt components, may not dissolve completely in a technical sense, as some undissolved material of a very small particle size may remain. Such small particles will generally be undetectable to the consumer.

As defined above, the edible films of the present invention comprise a phosphoprotein preparation, a source of one or more physiologically acceptable cations, and a plasticizer.

The phosphoprotein preparations suitable for use in the edible films of the present invention may be obtained by partially cross linking a partial hydrolysate of casein or a caseinate, that is, casein or a caseinate in which some, but not all, of the peptide bonds have been hydrolyzed.

As used herein, the term "cross linking", when used in the context of partially cross linking a partial hydrolysate of casein or a caseinate, means the formation of intermolecular covalent bonds between the amino acid residues of the casein molecules and/or casein molecule fragments comprising the partial hydrolysate. Preferably, the intermolecular covalent bonds comprise bonds between glutamine and lysine residues, ie glutamyl/lysyl covalent bonds. It will also be appreciated that some intramolecular cross linking, ie between amino acid residues on the same casein molecule or casein fragment, is likely to occur.

The term "partial", when used in the context of "partial cross linking", means that not all of the amino acid residues are cross-linked, ie that some non-cross linked amino acid residues will remain following the cross linking reaction.

The degree of partial cross linking is expressed herein in terms of micromoles of cross links per gram of protein.

The casein used to prepare the partial hydrolysate may be in any form; acid casein, rennet casein or a caseinate may all be used. Although chemical hydrolysis is by no means excluded, it is preferred that the partial hydrolysis is carried out enzymatically, in aqueous solution.

Suitable enzymes for performing the partial hydrolysis include proteases, such as trypsin and chymotrypsin. It is however particularly preferred that the enzyme used is trypsin, such as bovine derived or porcine pancreatic trypsin.

The partial hydrolysis should be carried out at a temperature and pH appropriate to the enzyme being used. For example, if bovine derived trypsin is used, the partial hydrolysis may

conveniently be carried out at a pH of between about 7 and about 8, and at a temperature of about 37°C. It will also be appreciated that at this pH, the casein will be present as a caseinate, eg sodium caseinate, depending on the buffer used in the reaction solution.

The reaction should be carried out for a sufficient period of time and under appropriate conditions, eg enzyme and casein concentrations, to allow the casein to be partially hydrolyzed, ie for some, but not all, of the peptide bonds to be hydrolyzed. When partial hydrolysis has been achieved, the reaction may conveniently be terminated, or at least substantially terminated, by inactivating the enzyme, for example by heating the reaction mixture to a temperature which will denature the enzyme, eg to about 80°C. It is not critical that the hydrolysis reaction be completely terminated, provided the partial cross linking reaction (as discussed below) is commenced once the desired degree of hydrolysis has been achieved. That is, a minor amount of hydrolysis may still continue while the partial cross linking reaction is being carried out.

It is generally preferred that the level of partial hydrolysis of the casein is in the range of about 0.5% to 10%, ie 0.5% to 10% of the peptide bonds in the casein are hydrolyzed. It is more preferred that the level of partial hydrolysis is in the range of about 2% to about 8%, such as about 3% to about 6%.

The degree of hydrolysis may be determined by methods known to those skilled in the art, conveniently by the TNBS (2, 4, 6 trinitrobenzene sulfonic acid) method.

It is also preferred that the degree of hydrolysis is such that about 15% or less of the casein or caseinate is rendered insoluble at pH 7, by the partial hydrolysis.

Purely by way of example, a partially hydrolyzed casein suitable for use in the films of the invention may be prepared by first solubilising a 10% isoelectric precipitated casein solution with NaOH to pH 7 at 50°C. The solution is then cooled to 37°C, and a porcine pancreatic trypsin preparation added at about 0.01% w/w casein and incubated for 15 minutes. A suitable porcine pancreatic trypsin preparation is that commercially available as Novo.4500K, molecular weight 23,400 Da, activity 4500 USP units/mg. Once the partial hydrolysis has been carried out, enzyme inactivation may be achieved by heating to 80°C and holding for 5 minutes.

Once the partially hydrolyzed casein has been prepared, it is then partially cross linked to form a phosphoprotein preparation suitable for use in the films of the present invention.

The partial cross linking may be carried out either enzymatically or chemically. It is however preferred that the partial cross linking is carried out enzymatically, conveniently using either of the enzymes lysyl oxidase or transglutaminase.

It is particularly preferred that the enzyme transglutaminase is used, and that the partial cross linking reaction is carried out at a pH of between about 7 and about 8. The reaction is carried out under suitable conditions and for a time sufficient to allow the desired degree of cross linking to take place. It is preferred that the reaction be carried out under conditions such that the degree of cross linking in the resulting phosphoprotein preparation comprises about 10 μmol or more cross links per gram of protein, more preferably between about 10 and about 250 μmol/g protein, more preferably between about 50 and 160 μmol/g protein, such as between about 110 and 150 μmol/g protein.

The degree of partial cross linking, in terms of the quantity of glutamyl/lysl bonds, may conveniently be determined by high performance liquid chromatography (HPLC), by carrying out a proteolytic digestion of the cross-linked proteins using suitable enzymes, conveniently pronase, leucine aminopeptidase, prolidase and carboxypeptidase, followed by HPLC of the proteolytic digest and quantification of the ε- (γ -Glutamyl)lysine (G-L) peak.

Again, once the partial cross linking reaction has been carried out, the reaction may conveniently be terminated by inactivation of the enzyme, typically by heating the reaction mixture to a temperature sufficient to denature the enzyme, for example to about 80°C for about 5 minutes. It is also generally preferred that, following completion of the partial cross linking and deactivation of the enzyme, the resulting phosphoprotein-containing solution is dialyzed or diafiltered to remove any remaining low molecular weight peptides and salts, conveniently using a membrane with a molecular weight cutoff of from about 10,000 Da to about 14,000 Da. The purified phosphoprotein-containing solution may be freeze dried or spray dried to obtain the phosphoprotein preparation in a solid form.

Any commercially available source of transglutaminase may be used to carry out the partial cross linking reaction. By way of example, a suitable enzyme is a 1% transglutaminase preparation commercially available from Ajinomoto Co. as Activa MP.

Purely by way of example, a phosphoprotein preparation suitable for use in the films of the present invention may be obtained by treating a partially hydrolyzed casein (prepared as described above) with a transglutaminase preparation (Activa MP) added at a ratio of 4.5% w/w casein and incubating the reaction mixture at 40°C for 18 hours.

Alternatively, the plastein reaction (which is an enzymatic reaction known to those skilled in the art) may be used. The cross linking may also be carried out between tyrosine residues using peroxidase and hydrogen peroxide.

As discussed above, although it is preferred that the partial cross linking is carried out enzymatically, partial cross linking by chemical means using a suitable reagent is by no means excluded. For example, a bifunctional aldehyde such as glutaraldehyde may be used.

The applicants have surprisingly found that the phosphoprotein preparations as described above are able to bind and thus solubilise significant quantities of cation salts, particularly salts of calcium and other divalent cations. Moreover, the phosphoprotein preparations have a higher solubility than unmodified casein. The applicants have further surprisingly found that by combining the phosphoprotein preparations with a source of a cation, the solubility of the phosphoprotein preparation can be controlled in a way which renders it capable forming a stable, thin film having the desirable properties discussed above. Without wishing to be bound by any theory, it is believed that by adding a source of a cation, such as a calcium salt, the water-binding capacity of the phosphoprotein is decreased, thereby enabling the formation of a thin, stable film which dissolves rapidly in the mouth.

The films of the invention therefore include a source of one or more physiologically acceptable cations. It is preferred that the cation is divalent, and is preferably selected from the group consisting of calcium, zinc and magnesium ions. The source of the cation may conveniently be an inorganic or organic salt, such as a phosphate, citrate or chloride. It is however preferred that the salt is a salt that is substantially insoluble in water.

It is particularly preferred that the cations are calcium ions. The source of calcium ions preferably comprises calcium phosphate, more preferably in the form of calcium hydroxyapatite. In particularly preferred embodiments, the source of calcium ions comprises natural milk calcium, such as that available from New Zealand Milk Products Ltd under the trade name ALAMIN ®, which comprises calcium, phosphate, and also protein, lactose, fat, moisture, sodium, potassium and chloride, in the following typical proportions:

Total mineral content 70% (w/w):	28% calcium and 48% phosphate
Protein (N x 6.38)	7%
Lactose	4%
Fat	1%
Free moisture	3%
Bound moisture	8%
Calcium	28,000 mg/100 g
Phosphorus	16,000 mg/100 g
Sodium	400 mg/100 g
Potassium	300 mg/100 g
Chloride	100 mg/100 g

In natural milk calcium phosphate, the calcium phosphate is generally regarded as being in the form of calcium hydroxyapatite. Natural milk calcium phosphate may be obtained by methods known in the art, typically by clarifying and pasteurizing acid whey permeate, cooling and ultrafiltering the permeate, followed by heating, pH adjustment and holding at the elevated temperature such that the minerals including calcium phosphate will be precipitated, and recovery of the precipitate.

Another suitable source of natural milk calcium phosphate is the product available under the trade mark Lactoval® from DMV International.

Alternatively, calcium phosphate in the form of tricalcium phosphate may be used.

Embodiments of the invention in which the source of calcium ions comprises natural milk calcium phosphate are particularly preferred. These preferred films demonstrate accelerated dissolution and getaway in the mouth. Without wishing to be bound by any theory, it is believed that the insoluble portion of the natural milk calcium phosphate may also act in a filler capacity, helping weaken the structure of the film and thus aid disintegration.

In other embodiments, the films may comprise another calcium source, such as calcium citrate or calcium chloride. In these embodiments it is generally preferred that the films also include a filler, such as talc.

The relative proportions of the source of cations and the phosphoprotein preparation in the edible films of the present invention will vary, depending on a number of factors. These include the properties, notably the solubility and cation-binding properties, of the particular phosphoprotein preparation being used, the type of cation and salt being used, and the desired properties of the edible film in question, such as its desired thickness and solubility. These in turn will depend on the intended end use of the film, for example whether it is to be used as a stand-alone film for delivery of breath-freshener, flavor or other active agent, or whether it is to be used for some other application such as an edible coating or packaging for a food.

However, in general terms, the calcium or other cation source should be included in the film formulations in an amount sufficient to aid in the formation of a flexible and cohesive film. The optimum amount of cation to be included may be readily determined by a person skilled in the art without undue experimentation.

By way of example, in embodiments of the invention in which the cation is calcium and the cation source is natural milk calcium phosphate as described above, the ratio (w/w) calcium ions:phosphoprotein may be in the range of about 0.05:1 to about 0.0136:1; the ratio (w/w) hydroxyapatite:phosphoprotein may be in the range of about 0.2:1 to about 0.92:1, and the ratio (w/w) natural milk calcium phosphate:phosphoprotein may be in the range of about 0.3:1 to about 1.3:1.

The edible films of the present invention may also desirably include a plasticizer (softener). The plasticizer may be any plasticizer suitable for inclusion in an edible film, such as a polyhydric alcohol, for example glycerol, sorbitol (preferably in combination with glycerol), polyethylene

glycol, or propylene glycol, or hydrogenated starch hydrolysates or corn syrups. In preferred embodiments the plasticizer comprises glycerol.

The plasticizer may conveniently constitute between about 10% and 25%, more preferably between about 15 to 18%, of the dry weight of the film.

Although not critical, the edible films of the present invention may also desirably include one or more emulsifiers. The emulsifiers may be any suitable natural or synthetic food-grade emulsifiers. For example, the emulsifier can include mono- and diacyl glycerides, citric acid esters of mono- and diglycerides, partially hydrogenated vegetable oils, lecithin, fatty acids, polyglycerol esters, polyethylene sorbitan esters, propylene glycol, sorbitan monopalmitate, sorbitan monostearate, or sorbitan tristearate, and other like emulsifiers.

The emulsifier(s) may conveniently constitute between 0.5% and 3% of the dry weight of the film.

Depending on the intended application of the film, it may also include one or more additional agents such as flavorings or breath freshening agents, sweeteners, coloring agents, pH control agents and stabilizers.

Flavoring or breath freshening agents that may be used may include, for example, essential oils, synthetic flavors or mixtures including oils derived from plants and fruits such as citrus oil, fruit essences such as orange, peppermint oil, spearmint oil, other mint oils, clove oils, oil of wintergreen, anise and the like, flavor oils with germ killing properties such as menthol, eucalyptol, eugenol, thymol, and germ killing agents like that found within the brand name product Listerine®, and combinations thereof. The amount of flavoring or breath freshening agent added will depend on the properties of the particular agent, but in general the flavoring or breath freshening agent may constitute between about 2% and 10% of the dry weight of the film

Suitable sweeteners include natural sweeteners such as sucrose, dextrose and maltose, and artificial sweeteners such as sucralose, aspartame, and salts of acesulfame such as Ace K.

Suitable colorings include food colors and dyes suitable for food applications, such as FD&C lakes and dyes.

Examples of pH control agents include orally acceptable buffers.

In certain embodiments, the edible film may include one or more active agents such as an oral care agent, a salivary stimulant agent, a pharmaceutical or veterinary agent, a nutraceutical agent, a vitamin, a mineral, and combinations thereof.

Oral care agents that may be incorporated into the films of the present invention include phosphates and fluorides, and anti-plaque/anti-gingivitis agents such as chlorohexidine, cetylpyridinium chloride and triclosan; and salivary stimulants.

Pharmaceutical, veterinary and nutraceutical agents, and vitamins and minerals which may be incorporated within the edible film compositions of the present invention include those agents which are suitable for oral consumption and can be placed within film formulations.

For example, the films may be used as delivery vectors for vaccines, antibiotics, analgesics, antihistamines, bacteriocins or local anaesthetics such as Novocain. The films may also be used to deliver therapeutics orally to small animals and other animals such as cows, pigs, deer and horses.

In general terms, the edible films of the present invention may be prepared by processing a mixture comprising a phosphoprotein preparation as described above, a source of one or more physiologically acceptable cations and a plasticizer, in a suitable manner to form a film; and, optionally, drying the film to reduce the moisture content to a desired level.

In preferred embodiments, the mixture includes water.

In some embodiments, the films may be prepared by casting. The method of preparation may conveniently comprise the steps of combining the dry ingredients of the film formulation with a blend of the water and other liquid ingredients. It is preferred that the dry ingredients are blended together before being added slowly to the blend of the liquid ingredients. When preparing the

blend of the liquid ingredients, it may also be desirable to heat this gently to assist in dissolving of the emulsifier (if present). The ingredients should be mixed thoroughly. It may be desirable to add further water to adjust the consistency of the solution during mixing, in order to obtain a solution of a consistency that can be spread onto a support for drying, and/or to adjust the thickness of the film. The solution may optionally be emulsified and/or degassed.

The pH of the solution should preferably be maintained at about 6 to about 8, more preferably at about 7.2 to 7.8, and still more preferably at about 7.5. This may conveniently be achieved by adding a suitable pH control agent, such as sodium hydroxide.

The solution may then be poured or otherwise applied on to a suitable support, such as a dry glass plate, or to a surface on which it is intended to form a coating. The films of the present invention may be either self-supporting, or they will adhere when hot to a structural surface such as paper, and thus may serve as a coating if desired.

The films may be dried in any suitable manner, including air-drying, oven drying, reflectance window drying or roller drying.

In alternate preferred embodiments, the films of the present invention may be prepared by extruding a mixture comprising the film ingredients. In this embodiment, it may not be essential to include a drying step following extrusion of the film, depending on the proportion of water that is included in the extrusion mixture and the final moisture content of the extruded film. The films may conveniently be prepared by combining the liquid ingredients, slowly adding them to a mixture of the dry ingredients, adjusting the pH (for example to about 7.5) so that the mixture forms a thermoplastic mass, cooling the mixture so that the mass becomes crumbly, and grinding the mass to a powder which is suitable for extrusion.

The desired thickness of the films on the present invention will vary, depending on the intended use of the film. However, the thickness of edible films of the invention may desirably be in the range of about 30 to about 80μm, such as about 40-50 μm, for example around 43 to 47 μm. The thickness of the film may be controlled by adjusting various parameters, including the proportion of plasticizer, the proportion of water in the dry product, the method used for spreading and drying, the initial thickness of the wet film, and, to a certain extent, the pH.

The invention will now be explained in more detail with reference to the following examples, which are given for illustration of the invention and are not intended to be limiting thereof.

EXAMPLES

Example 1

Ingredients	*%*	*adjusted %*[1]
Phosphoprotein (MAP112)	30	18.75
Alamin®	30	18.75
Ace-K	1	0.625
Emulsifier - Lamegin 609ZE	1.5	0.935
Flavor - Peppermint Oil	6	3.75
Glycerol	15	9.38
Water	16.5	47.8

[1]Extra water was added to solutions to enable spreading on glass plates for drying.

Method of preparation:

The dry ingredients (phosphoprotein, Alamin®, and Ace-K) were blended. The phosphoprotein was prepared as described in Example 3.

The liquid ingredients (emulsifier, flavor, glycerol and water) were blended. The mixture was heated gently to 30-40°C as required, to dissolve the emulsifier.

The dry ingredients were added slowly to the liquids, stirring until all ingredients were well mixed. Extra water was added to solutions to enable spreading on glass plates for drying.

The pH was adjusted to 7.5 with 4M NaOH.

The solution was spread onto a dry glass plate (0.25mm thickness), and dried at 100-110°C for about 4-5 minutes. The plate was left to rehydrate at room temperature, then the film peeled off the plate using blade.

Example 2

Ingredients	%	*adjusted %*[1]
Phosphoprotein (MAP112)	30	18.75
Alamin	30	18.75
Ace-K	1	0.625
Emulsifier - Lamegin 609ZE	1.5	0.935
Flavor - Peppermint Oil	6	3.75
Glycerol	13	8.125
Water	18.5	49.065

[1]Extra water was added to solutions to enable spreading on glass plates for drying.

An edible film was prepared using the preparative method as described above for Example 1.

Example 3

Preparation of a phosphoprotein (MAP 112)

Tryptic hydrolysis

Fifty-five kilograms of sodium caseinate was dispersed in 50°C deionised water so that a final concentration of 522L was achieved. The solution was cooled to 37°C, the pH adjusted to 7.06 with NaOH and porcine derived trypsin (Novo.4500K, molecular weight 23,400 Da, activity 4500 USP units/mg) added at 0.01% w/w casein and incubated for five minutes. The solution was heated to 80°C over 15 minutes, held for four minutes and cooled to 45°C.

The molecular weight profiles of the partially hydrolysed preparation was determined by gel filtration as follows. A 1% protein solution was prepared in 6M Urea, with 50mM sodium phosphate at pH 7.5 as the buffer. This solution was centrifuged at 10 000 x g for 10 minutes and passed through a 0.2μm filter. A sample volume of 500 μl injected into the 100μl sample loop of

a Pharmacia FPLC fitted with a Superdex 200 10/30HR column. The running buffer used was 6 M Urea, with 50mM sodium phosphate at pH 7.5 and flow rate of 0.5 ml/min. Detection was by UV absorption (280 ηm). The protein absorption curve was integrated and arbitrarily divided into the following four molecular weight groupings:

1) greater than about 30,000 Daltons
2) less than about 30,000 Daltons and greater than about 21,000 Daltons
3) less than about 21,000 Daltons and greater than about 12,000 Daltons
4) less than about 12,000 Daltons.

Molecular weight profile after hydrolysis

Molecular weight range	Percentage
≥30,000	10.7
<30,000, ≥ 21,000	57.8
<21,000, ≥ 12,000	15.7
<12,000	15.8

Transglutaminase treatment

The pH was re-adjusted to 7.0, and transglutaminase (1% commercial preparation, Activa MP, Ajinomoto Co) added at a ratio of 4.5% w/w casein and incubated for 15 hours (temperature at the end of incubation was 32°C). The solution was heated to 80°C and held for 5 minutes. The solution was diluted to 1000L and cooled to 5°C. The solution was ultrafiltered until a final concentration of 20% solids was achieved and then spray dried. The molecular weight material greater than 30,000Da was increased by 100% and the number of cross-links in the protein was 92μmol/g.

Example 4

Preparation of extruded films

Extruded films were prepared using the following formulation on a Brabender Twin Screw extruder.

Ingredients	*Extrusion 1*	*Extrusion 2*
Protein MAP112 (Example 3)	32.40	30.0
Milk derived calcium phosphate	32.40	30.0
Ace-K	0.99	1.0
Emulsifier	1.62	1.5
Flavour Peppermint Oil	6.48	6.0
Glycerol	16.20	13.0
Water	9.91	18.5
	100.0	100.0

Method of Preparation

The liquid ingredients (water, glycerol, peppermint oil and emulsifier) were mixed and slowly added to the dry mix (Protein MAP112, Milk derived calcium phosphate and Acesulfame K) and stirred until all ingredients were well mixed. The pH was adjusted to 7.5 with 4M NaOH. The resulting mixture formed a thermoplastic mass that was crumbly at temperatures below 18°C. The mixture was cooled to 0°C and ground to a powder.

Extruder Conditions

Feed hopper screw speed 5RPM

Extruder screw speed 8RPM

Barrel temperature profile (from feed end)

Section 1 50°C

Section 2 60°C

Section 3 65°C

Section 4 75°C

Nozzle 80°C

Nozzle aperture 100μm

The crumble was fed into hopper and extruded as a thin film. As the film was exited the nozzle it was picked up by strips of paper (silicon backed, 0.25μm thickness 2m length). The paper speed was varied to achieve films with thicknesses ranging from 40μm to 150μm. The films were packed in foil laminate bags.

Example 5

Determination of degree of cross linking using High Performance Liquid Chromatography

Chemicals and Reagents

ε- (γ -Glutamyl)lysine (G-L) and trifluoroacetic acid (TFA, protein sequencing grade), Prolidase (porcine kidney), Leucine aminopeptidase and cytosol from hog kidney, Carboxypeptidase A (bovine pancreas) and Pronase (*Streptomyces griseus*) and TRIS [Tris(hydroxymethyl)aminomethane] were purchased from Sigma Chem. Co. (Sydney, Australia). Acetonitrile (HPLC grade) was purchased from Biolab (Christchurch, New Zealand).

Proteolytic digestion of the cross-linked proteins

A 48—50 mg aliquot of protein sample was weighed in a glass test tube (total volume 15mL). A crystal of thymol and 2 ml of 0.2 M Tris (pH 8.0, HCl) was added, the solution vortexed and then incubated at 40°C for 1 h to allow the dispersion of the protein. An aliquot of Pronase (0.4 U/mg protein) was added to the mixture which was then incubated at 37°C for 24 h. The pronase digestion was continued for a further 24 h by the addition of another equal sized aliquot of pronase. After inactivation of pronase by heating at 100°C (waterbath) for 10 min, the digestion was continued adding leucine aminopeptidase (0.4 U/mg protein), the solution being treated as for the pronase incubation. The digestion was continued using prolidase (0.45 U/mg protein) and then carboxypeptidase A (0.2 U/mg protein). After final inactivation the mixture was diluted to 7.5 g with ultrapure water (MilliQ water purification system)(Millipore, North Ryde, Australia).

HPLC analysis of G-L

The HPLC system consisted of a Dionex GP40 gradient pump solvent delivery system connected to an ICI Instruments 1210 UV/Vis detector. Data was captured via an ITNS Acquisition Board and analysed using the AZUR chromatography software Version 1.1. The samples (100 µl) were separated on an Inertsil ODS-2 column (5 µm, 150 x 7.6 mm) (Phenomenex, Auckland, New Zealand) connected to a guard column (C_{18} ODS, 4 mm x 3.0 mm ID)(Phenomenex) and a 2 µm prefilter. Analysis was performed at 2.5°C. The mobile phases were 0.1% TFA (v/v)(solvent A) and acetonitrile containing 0.1% TFA (v/v) (solvent B). The solvent program was as follows: 100 % solvent A for 20 min, 0% to 100% solvent B from 20 to

25 min, 100 % solvent B from 25 to 50 min. The detector wavelength was set at 210 nm and the flow rate was 1.0 ml/min.

All samples from proteolytic digestion were filtered on a 0.45 µm Millex-HA Millipore filter unit. 200µl of sample was mixed with 100 µl of distilled water and 100µl TFA (2%w/w). The G-L peak was identified by comparison to elution time of a standard and confirmed by standard addition of a G-L standard solution to the sample.

Example 6

	5A	**5B**	**5C**
Ingredients		*% weight/weight*	
Phosphoprotein			
Lot2/18	22.5	-	-
MAP106	-	30	-
MAP109	-	-	30
Milk calcium phosphate	22.5	10	30
Ace-K	0.75	1	1
Emulsifier - Lamegin 609ZE	1.13	1.5	1.5
Flavor - Peppermint Oil B&J	4.5	6	6
Glycerol	7.5	18	15
Water	41.12	33.5	16.5

Method of preparation:

The dry ingredients (phosphoprotein, Alamin®, and Ace-K) were blended.

The characteristics of the phosphoproteins used in the preparations were as follows: Lot 102: degree of hydrolysis (DH) 8.8%, degree of cross linking 150µmol/g protein; MAP 106: DH 4.4%, degree of cross linking 155µmol/g protein, MAP 109: DH 5.7%, degree of cross linking 155µmol/g protein.

The liquid ingredients (emulsifier, flavor, glycerol and water) were blended. The mixture was heated gently to 30- 40°C as required, to dissolve the emulsifier.

The dry ingredients were added slowly to the liquid ingredients, stirring until all ingredients were well mixed.

The pH was adjusted to 7.5 with 4M NaOH.

The solution was spread onto a dry glass plate (0.25mm thickness), and dried at 100-110°C for about 4-5 minutes. The plate was left to rehydrate at room temperature and then the film peeled off the plate using a blade.

Description of Films

Lot2/18 and MAP106 made flexible, slightly sticky films that dissolved quickly. MAP109 was also very flexible, tasting slightly damp and clung briefly to the tongue before quickly dissolving. All films also gave an intense flavour burst.

Example 7

	6A	6B	6C	6D	6E
Ingredients			*% weight/weight*		
Phosphoprotein (MAP112)	30	27	27	22.5	23.1
Salts					
$CaCl_2$	3	-	-	-	-
$CaCO_3$	-	13.6	-	-	-
Ca Citrate	-	-	13.6	-	-
Ca_3PO_4	-	-	-	27.1	-
$MgCl_2$	-	-	-	-	23.1
Ace-K	1	0.9	0.9	0.8	0.8
Emulsifier - Lamegin 609ZE	1.5	1.4	1.4	1.1	1.2
Flavor - Peppermint	6	5.4	5.4	4.5	4.6
Glycerol	15	10.9	10.9	13.5	11.5
Water	43.5	40.8	40.8	24.9	35.8

Method of preparation:

The films were prepared as per Example 6.

Description of Films

All the films were thin, flexible and dissolved quickly. All films also gave an intense flavour burst. The carbonate, citrate and phosphate films were opaque in colour, while the chloride films were clear. The films containing a chloride salt were slightly sticky.

Example 8

Method of determining degree of hydrolysis of partially hydrolysed casein

Samples were prepared as either 0.1% or 1% protein (w/v) solutions in distilled water. A 100µl aliquot of sample was added to 800µl of 0.2125 M sodium phosphate buffer, pH 8.20. To this 800 µl 0.1% TNBS reagent was added, and the reagents were mixed well, wrapped in foil and incubated at 50°C in a covered water-bath. After exactly 60 minutes the reaction was terminated by the addition of 1600 µl of 100mM HCl and the samples left to cool to room temperature for 30 minutes before reading absorbance against a buffer/TNBS blank at 340 ηm.

INDUSTRIAL APPLICATION

As described above, the films of the present invention have a number of applications. For example, they may be used to deliver a breath freshener or other oral care agent. Alternatively or additionally, they may act as a carrier for a pharmaceutical or veterinary agent, a nutraceutical agent, a salivary stimulant agent, a vitamin, a mineral, or combinations thereof. In other applications, the edible films of the present invention may be used as a readily soluble and/or edible coating for foods such as fruit or confectionery products. They may also be used in food product labeling, such as cheese labeling. A further application is in the preparation of novel confections such as tongue tattoos.

In still other applications, the films may be used to as a vehicle to apply active agents topically to the skin. For example, sunscreen (SPF) agents, burn recovery agents, Herpes simplex remedies, and topical antifungal disease agents may be incorporated in the films of the present invention.

In still further applications, the films may be used in diagnostic kits, particularly in immunology, or in forensic applications.

At least in the preferred embodiments, the edible films of the present invention have the advantage of "grabbing" less in the mouth and providing a relatively clean mouth feel on dissolution, as compared to certain currently available pullulan-based films. The quick getaway in the mouth of the films of the present invention also gives them potential for use in sudden flavor release.

While the invention has been described in detail and with reference to particular embodiments thereof, various changes and modifications of these embodiments will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the scope and scope of the present invention as defined in the appended claims.